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Treatment of hepatitis B virus-associated membranous nephropathy with adenine arabinoside and thymic extract

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Treatment of hepatitis B virus-associated membranous nephropathy with adenine arabinoside and thymic extract. Previously we found that corticosteroid treatment in the hepatitis B virus (HBV)-associated membranous nephropathy (HBVMN) was not associated with a favorable outcome. To distinguish the differences of the HBV DNA in macrophage, T and B cells among HBVMN patients with or without corticosteroid treatment, serial studies at different time points were investigated. HBV DNA appeared as an "episomal" molecule as with 3.2 kb in macrophage, T and B cells. This molecule disappeared after 12 months among HBVMN patients without corticosteroid treatment. HBV DNA, by contrast, appeared as episomal form even three years later in T cells, with frequent proteinuria among HBVMN patients with corticosteroid treatment. This finding indicates that the use of corticosteroids leads to a potential risk of enhancing HBV viral replication in T cells. We studied 24 HBVMN patients who had previously received corticosteroid treatment and had persistent proteinuria, who were administered combination therapy with adenine arabinoside for two weeks and thymic extract (Thymostimulin) for six months to decrease urine protein loss and obtain seroconversion. These 24 patients had heavy (22 of 24, 91.6%) or mild (2 of 24, 8.4%) proteinuria prior to adenine arabinoside and thymostimulin treatment. All 24 patients demonstrated HBV DNA in mononuclear cells and simultaneously exhibited sera positive with HBsAg and HBeAg. In contrast, after treatment only one case (4.2%) had heavy and two cases (8.4%) mild proteinuria; HBV DNA was demonstrated in macrophage (4 of 24, 16.7%), T cells (9 of 24, 37.5%), and B cells (6 of 24, 25%) as well as serum (24 of 24, 100%) prior to treatment. The decreases to 16.7%, 37.5%, 25% and 41.6% in the macrophage, T cell, B cell, and serum respectively, were statistically significant ($P < 0.01$) in each instance. In addition, six cases with complete remission of proteinuria changed their hepatitis B markers. Four cases (16.7%) changed from HBe (+)/anti-HBe (−) to HBe (−)/anti-HBe (−). These results suggest that combination therapy of adenine arabinoside and Thymostimulin in HBVMN patients is more effective in the improvement of proteinuria than corticosteroid treatment.

Human infection with hepatitis B virus (HBV) can lead to acute and chronic hepatitis, liver cirrhosis and hepatocellular carcinoma [1–3]. Combes et al also described the association of HBV with membranous nephropathy (HBVMN) [4]. Current evidence suggests that HBV itself is not cytopathic and that liver cell damage is limited to the host immune responses directed at viral and/or self-antigens expressed on the membrane of infected hepatocytes [5, 6]. Recent reports also indicate that HBV DNA may be detected in peripheral blood leukocytes of patients with HBV infection [7, 8]. If HBV does infect human leukocytes, such infection may interfere with the immunological functions of the mononuclear cells, thus playing an important role in the pathogenesis of HBV-induced liver or kidney diseases. Most of the studies confirming that HBV DNA is detectable in human T cells, however, were carried out on chronic HBV liver disease or HBV asymptomatic carriers [7, 8]. Only one study previously has demonstrated the presence of HBV DNA in macrophages [9].

The HBVMN is transmitted horizontally [10, 11]. The macrophage acts as the first line defense mechanism and provides antigen recognition. HBV DNA, therefore should be detectable in macrophage at the early stage of HBV infection among HBVMN patients. Direct detection of HBV DNA in macrophages, T and B cells, and serum using the molecular hybridization test has been proven to be a sensitive and reliable method in determining viral replication and infectivity of HBsAg carriers [12–14]. On the other hand, correlation between hepatitis B e antigen (HBeAg) and antibody (anti-HBe) and HBV DNA in HBVMN has not been well documented. The specific aim of this longitudinal study, therefore, was to investigate the serial changes of HBV DNA in macrophage, T, B cells, serum and HBV serological markers (HBsAg, HBeAg) and their association with proteinuria involved in HBVMN patients before and after treatment.

Methods

Patient selection

Twenty-four children younger than 12 years of age diagnosed with HBVMN from January 1986 to November 1987 were recruited for the study and referred to the Department of Pediatrics, Veterans General Hospital for renal biopsy. Inclusion criteria consisted of treatment of prednisolone 2 mg per kg daily for more than eight weeks and persistent nephrotic syndrome or heavy proteinuria. In each HBVMN case, a kidney biopsy specimen was collected and divided into three portions for light, immunofluorescence, and electron microscopic studies [10, 11]. HBsAg was stained with polyclonal rabbit anti-HBs (Dako Corporation, Santa Barbara, California, USA) and monoclonal mouse anti-HBs IgG antisera by indirect immunofluorescent technique [10, 11]. For the immunofluorescent demonstration of HBcAg in tissues, 4 μm thick cryostat

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was first stained with 1:1600 dilution of rabbit anti-HBc antibody (Dako Corp), and HBe was stained with FITC-conjugated anti-HBe IgG monoclonal antibody [15]. For negative control, anti-HBe, anti-HBs and anti-HBc antibodies were replaced by phosphate buffered saline (PBS). Ten cases of membranous nephropathy (MN) in adult patients with no detectable serum HBsAg (9 cases) or positive serum HBsAg (1 case) were also examined. A negative result was obtained in all.

The criteria for diagnosis of HBVMN were: (1) renal biopsy showed membranous glomerular thickening in the light microscopy, and immunofluorescent staining demonstrated at least one of the diffuse granular capillary membranous deposits among HBsAg, HBcAg and HBeAg in immunohistology; (2) serum HBsAg positive; (3) abnormal urinalysis; (4) there were no other types of viral infection such as cytomegalovirus or Epstein-Barr virus. The criteria of nephrotic syndrome (NS) include: (1) heavy proteinuria greater than 40 mg/M^2/hr determined quantitatively on a 24-hour overnight urine collection; (2) hypoalbuminemia less than 2.5 g/dl; (3) puffy face, generalized edema. Any patient with only the first criterion was defined as "heavy proteinuria". Proteinuria between 4 mg/M^2/hr and 40 mg/M^2/hr was defined as "mild proteinuria". Less than 4 mg/M^2/hr was defined as "free of proteinuria".

After renal biopsy confirmed HBVMN, these HBVMN patients received one course of intravenous infusion of 15 mg per kg body weight daily of adenine arabinoside (Ara-A) for two weeks. They then received thymic extract (Thymostimulin, TP-1, Instituto Farmacologico Serono, Rome, Italy) 2 mg per kg body weight intramuscular daily for six months. If a patient had seroconversion to HBsAg (−)/HBe (−) then Thymostimulin therapy was stopped.

Control subjects

The control groups included 10 age- and sex-matched HBsAg (−)/HBe (−) healthy controls.

Serologic tests

Sera separated soon after venesection were stored at −70°C. Serum HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc were measured by radioimmunoassay (Abbott Laboratories, Abbot Park, Illinois, USA).

Separation of macrophage, T and B cells

Heparinized peripheral blood samples were obtained monthly from the HBVMN patients. The blood mononuclear cells (MNC) were isolated by the Ficoll-Hypaque gradient density method [10, 16]. Fifteen milliliters of MNC suspension were put into a 100 × 15 mm plastic petri dish (Flacon plastics) and incubated in a humidified, 37°C, 5% CO_2 incubator for 50 minutes. The adherent cells were harvested by rubber "policeman", washed, and then the whole process was repeated three times. The non-adherent cells were separated by E-rosettes technique and flow cytometry (Coulter EPICS C, Hialeah, Florida, USA). The T cells forming E-rosettes were pelleted on the bottom and were separated with cold distilled water. The B cells were separated with Ficoll-Hypaque gradient and centrifuged at 400 g at room temperature for 30 minutes. Then the interface layer was aspirated and passed through flow cytometry.

To verify the effectiveness of the separation procedure, T cells, B cells and macrophages were incubated for 30 minutes at 4°C with phycoerythrin-labeled monoclonal antibodies to CD3 (Ortho Pharmaceuticals, Raritan, New Jersey, USA), B7 (Ortho) and Mo (Coulter Immunology). T cells, B cells and macrophages bound to each monoclonal antibody were sorted with a fluorescence-activated cell sorter (Coulter EPICS C). Using this sorting technique, the T cell suspension was almost 100% CD3 positive cells and the B cell suspension about 100% B7-positive cells. The final cell suspension contained about 100% Mo2 and 98% peroxidase-positive cells.

DNA extraction

Macrophage, T and B cells were washed at least three times with phosphate-buffered saline (PBS; 150 mM NaCl, 10 mM phosphate, pH 7.2) to remove HBV which might be present in the serum. The supernatant from the last wash was kept to use as control in hybridization experiments. The washed cells were then digested overnight with 500 μg/ml of self-digested protease in 10 mM Tris, pH 8.0, 150 mM NaCl, 10 mM EDTA, and 0.5% sodium dodecyl sulfate (SDS) at 37°C. The mixture was then extracted twice with equilibrated phenol and once with phenol/chloroform/isoamyl-alcohol mixture (25:24:1). RNase A was added to 100 μg/ml, incubated at 37°C for one hour, and treated with protease at 500 μg/ml at 37°C for one hour. DNA was precipitated with two volumes of cold ethanol after further phenol extraction. The DNA pellet obtained after centrifugation was dissolved in 10.0 mM Tris, pH 8.0, and 10 mM EDTA. Concentration and quality of each DNA preparation were determined by measurements of A_{260} and A_{280} [17].

Hybridization

Thirty microliters of serum were first transferred to a fresh tube for detection by slot hybridization of HBV DNA in sera [13]. SDS and EDTA were added in 5 μl volume each to final concentrations of 0.5% and 5 mM, respectively; 5 μl of proteinase K (1.2 mg/ml) were added and the mixture was incubated at 56°C for four hours. A single volume of 2 M NaCl and two volumes of 1 M NaOH were then added. The solution was loaded onto nitrocellulose filters (Schleicher & Schuell, BA85, 0.45 μm, Dassel, Germany) using a 96-well manifold designed for multiple-sample dot hybridization (Schleicher & Schuell, SRC 072/0, minifold II) under mild suction after standing at room temperature for 20 minutes. Four volumes of neutralization solution (0.5 M Tris, pH 7.4, 3 M NaCl) were then used to wash the filter. The filter was baked at 80°C under vacuum and used for hybridization experiments. This method is able to detect less than 1 pg of HBV DNA.

Twenty micrograms of EcoRI-digested or undigested leukocyte DNA were electrophoresed in 0.8% agarose gel for Southern blot analysis of DNA samples. DNA was denatured by soaking the gel in several volumes of 1.5 M NaCl and 0.5 M NaOH for one hour at room temperature with constant shaking, followed by neutralization in 1 M Tris (pH 8.0) and 1.5 M NaCl for one hour at room temperature. Blotting of electrophoresed DNA samples onto nitrocellulose filter was carried out as described [18] for 36 hours. The nitrocellulose filter was then baked for two hours at 80°C under vacuum. The filters were soaked in 6× SSC (1× SSC is 150 mM NaCl, 15 mM Na_2 citrate), 0.5% SDS, 100 μg/ml salmon sperm DNA (Sheared and denatured), 10 mM EDTA, and 5× Denhardt's solution (1×

Denhardt's solution is 0.02% Ficoll, 0.02% polyvinylpyrolidone, 0.02% bovine serum albumin) at 68°C for six hours for prehybridization. The HBV probe was prepared from an HBV plasmid clone pTWL1 [19]. EcoRI digestion of pTWL1 released a 3.2 kb fragment, which was then freed from its cloning vector (pUC8) by isolation from low melting temperature agarose gel. The HBV fragment was labelled by nick-translation procedure to specific activity of 2 to 5 × 10^8 cpm/μg DNA using [α-^{32}P]dCTP (New England Nuclear, Boston, Massachusetts, USA). Alkali-denatured ^{32}P-labeled HBV DNA probe was added to the prehybridization solution after prehybridization. At least a tenfold excess of denatured and unlabeled pUC8 DNA was also added to the hybridization mixture in order to eliminate spurious hybridization signals due to possible minor contamination of the cloning vector (pUC8). The hybridization was carried out at 68°C for 36 hours. The filters were washed twice in 2× SSC, 0.1% SDS for 15 minutes each at room temperature, twice in 1× SSC, 0.1% SDS at 65°C for one hour each after hybridization, and finally in two changes of 0.5× SSC, 0.1% SDS, also at 65°C for one hour each. The filters were dried and autoradiographed with x-ray film (Eastman Kodak, X-MOATAR film, Rochester, New York, USA) at −70°C with two sheets of intensifying screens (KYOKKO) for various time intervals.

Statistics

Data are presented as mean ± SD. Comparisons were made using Chi-Square test and Student's *t*-test for paired data.

Results

HBV DNA in macrophage, T cells, and B cells among HBVMN patients with or without corticosteroid treatment

Macrophage, T cell and B cell cellular DNA samples were analyzed by southern blot hybridization using EcoR1 to determine the form of HBV DNA molecules. HBV DNA appeared as 3.2 kb episomal molecules only in macrophage, T and B cells before the first six months after onset of disease (Fig. 1A) for those HBVMN patients who had not received corticosteroid treatment. When HBV DNA was digested with BanH1, 1.4 kb molecules appeared. Comparison of HindIII digested and undigested DNA revealed that the position of the HBV band had not changed after HindIII digestion, indicating that the HBV DNA molecules of the HBVMN patients did not contain a HindIII site as reported by others [17]. Six months later, the 3.2 kb HBV DNA in macrophage had disappeared. The 3.2 kb HBV DNA persistently existed, however, either in the T or B cells. The cellular HBV DNA disappeared 12 months later. HBV DNA appeared by contrast as episomal form (3.2 kb) even three years later in T cells (Fig. 1B) in patients with persistent proteinuria who had received corticosteroid treatment. These results indicate that the use of corticosteroid may lead to a potential risk of enhancing HBV viral replication in T cells.

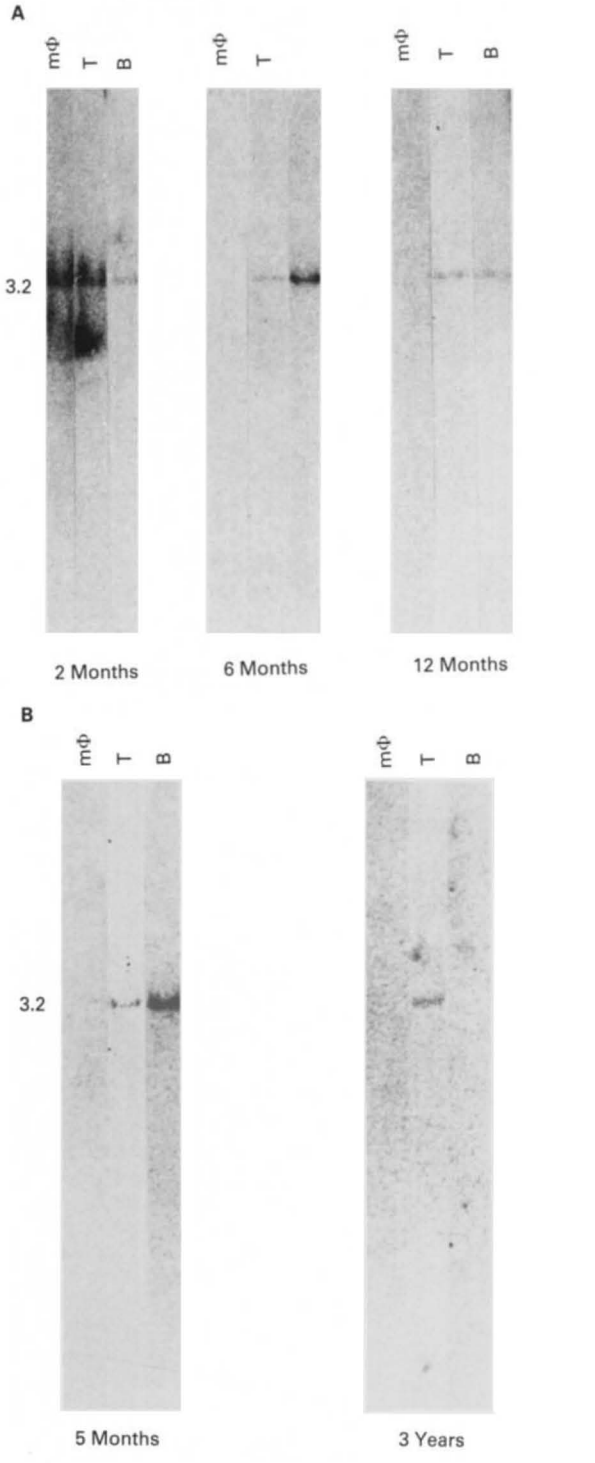

Fig. 1. *Serial HBV DNA in macrophage, T and B cells was observed in two cases (***A.** *without steroid treatment;* **B.** *with steroid treatment).*

Cellular HBV DNA profile in macrophages, T cells and B cells among 24 HBVMN patients

Cellular HBV DNA profiles in macrophages, T cells and B cells among the 24 HBVMN patients who had previously received corticosteroid treatment before the combination treatment of adenine arabinoside and Thymostimulin, using slot-blot hybridization are shown in Figure 2. Fifteen cases (62.5%), 23 cases (95.8%) and 21 cases (87.5%) demonstrated HBV DNA in macrophages, T cells and B cells, respectively. All cases at this time had HBV DNA in both sera and mononuclear cells simultaneously as shown in Table 1.

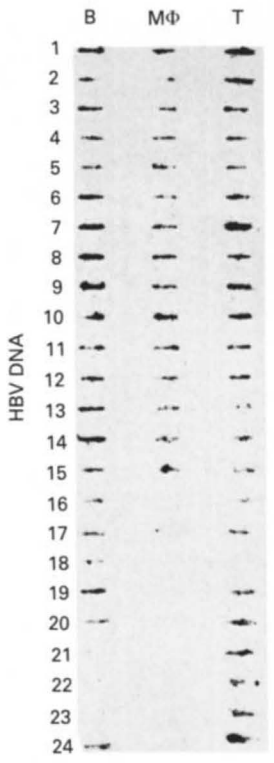

Fig. 2. *Study of cellular HBV DNA in 24 cases using slot-blot hybridization in the pre-treatment.*

Evaluation of adenine arabinoside and Thymostimulin treatments by serial detection of HBV markers and proteinuria at different time intervals

All cases (100%) had cellular HBV DNA in sera and in mononuclear cells (either in the macrophage or T and B cells) with 100% seropositivity rates of both HBsAg (+)/HBe (+) simultaneously, and before adenine arabinoside and Thymostimulin treatments. A review of the clinical manifestations demonstrated that 22 cases (91.6%) had heavy proteinuria and two cases (8.4%) mild proteinuria during the pre-treatment time (Table 1). By contrast, two cases with mild proteinuria recovered free of proteinuria, and all 22 cases of heavy proteinuria had decreased their urinary protein losses with only two cases still in the mild proteinuria state (Table 1). Twelve cases (50%), 20 cases (83.3%), 14 cases (58.3%) and 19 cases (79.2%) demonstrated HBV DNA in macrophages, T cells and B cells and plasma, respectively. Only one case, however, had HBeAg seroconversion with HBe (−)/anti-HBe (+), and one case only presented anti-HBe antibody in HBe (+)/anti-HBe (+) status. Only one case still had urinary protein loss more than 40 mg per M^2 per day, and two cases had mild proteinuria in the total of 22 cases of heavy proteinuria before treatment (Table 1), at the end of six months after treatment. The other cases attained remission. There were only four cases (16.7%) in macrophages, nine cases (37.5%) in T cells, and six cases (25%) in B cells with cellular HBV DNA. Ten cases (41.6%), however, were still positive with HBV DNA in sera as shown in Table 1. Therefore HBV DNA was detected more frequently in serum than in mononuclear cells. These striking decreases of cellular DNA from pre-treatment (62.5%, 95.8%, 87.5%, and 100%, respectively) to post-treatment (16.7%, 37.5%, 25.0%, and 41.6%) in macrophages, T cells, B cells, and plasma HBV DNA were statistically significant ($P < 0.01$) (Table 1). Serologically, six months after treatment, four cases (16.7%) changed their hepatitis B markers from HBsAg (+)/HBsAb (−), HBe (+)/anti-HBe (−) to HBsAg (+)/HBsAb (−), HBe (+)/anti-HBe (+), two cases (8.4%) from HBsAg (+)/HBsAb (−), HBe (+)/anti-HBe (−) to HBsAg (+)/HBsAb (−), HBe (−)/anti-HBe (+) and another two cases (8.4%) from HBsAg (+)/HBsAb (−), HBe (+)/anti-HBe (−) to HBsAg (−)/HBsAb (−), HBe (−)/anti-HBe (+) and HBsAg (−)/HBsAb (+), HBe (−)/anti-HBe (+), respectively; all had complete remission of proteinuria. The last heavy proteinuria case still progressively improved, becoming mild proteinuria at second month and then complete remission at eleventh month, after discontinuation of adenine arabinoside and Thymostimulin treatment. The other two mild proteinuria cases also attained complete remission at nine and ten months, respectively. During the second six follow-up months, one case changed from HBsAg (+)/HBsAb (−), HBe (−)/anti-HBe (+) to HBs (−)/anti-HBs (−), HBe (−)/anti-HBe (+), and another one from HBsAg (+)/HBsAb (−), HBe (+)/anti-HBe (−) to HBsAg (+)/HBsAb (−), HBe (+)/anti-HBe (+).

Correlation among HBV DNA, HBsAg, HBe markers and proteinuria

There were 22 cases (91.6%) in the heavy proteinuria state (>40 mg/M^2/hr) who were positive for both HBe (+)/anti-HBe (−) and cellular/serum HBV DNA (+) as shown in Table 2. On the other hand, either in the HBe (−)/anti-HBe (−) or HBe (−)/anti-HBe (+) status, only one case (6.25%) was positive for cellular/serum HBV DNA and had heavy proteinuria. The difference between these two groups was statistically significant ($P < 0.001$). This result also confirms our previous data that most of the cases had disappearance of heavy proteinuria and then attained HBe seroconversion [11]. Cellular/serum HBV DNA and proteinuria in contrast did not always disappear in the HBe (−)/HBsAg (+) status either with HBsAb (−) or HBsAb (+).

Discussion

In the present study we demonstrated that HBV DNA appeared as episomal molecule only with 3.2 kb in macrophages, T cells and B cells, but that this molecule disappeared after 12 months among HBVMN patients without corticosteroid treatment. By contrast, HBVMN appeared as episomal form up to three years later in T cells with frequent proteinuria among HBVMN patients with corticosteroid treatment, indicating that corticosteroids has a potential risk of enhancing HBV replication in mononuclear cells.

HBV DNA in mononuclear cells in the present study was detected by Southern blot analysis of cellular DNA extracted from extensively washed macrophages, T cells and B cells. The cells were washed at least three times in order to rule out the possibility of serum contamination of mononuclear cells. The supernatant from the last wash showed no detectable signals by dot hybridization. Furthermore, the HBV DNA probe preparations, when tested against pUC8 DNA on filter, showed no hybridization signals, indicating that the probe preparations

Table 1. Serial changes of hepatitis B markers, HBV DNA and proteinuria before and after Ara-A and TP-1 treatment

	Before Ara-A & TP-1	After Ara-A & TP-1			
		3rd Month	6th Month	9th Month	12th Month
Proteinuria					
>40 mg/M²/hr	22 (91.6%)[a,b,c]	2 (8.3%)[b,d]	1 (4.2%)[c,f]	0	0
4–40 mg/M²/hr	2 (8.4%)[a]	3 (12.5%)[e]	2 (8.4%)[g]	2 (8.4%)	0
<4 mg/M²/hr	0	19 (79.2%)[d,e]	21 (87.5%)[f,g]	22 (91.6%)	24 (100%)
HBV DNA (+)					
in Monoclonal antibodies	15 (62.5%)	12 (50.0%)	4 (16.7%)	0	0
T cell	23 (95.8%)	20 (83.3%)	9 (37.5%)	6	3 (12.5%)
B cell	21 (87.5%)	14 (58.3%)	6 (25.0%)	4	2 (8.4%)
in plasma	24 (100%)	19 (79.2%)	10 (41.6%)	7	5 (20.8%)
HBsAg(+)	24 (100%)	24 (100%)	22 (91.6%)	22 (91.6%)	21 (87.5%)
eAg(+)eAb(−)	24 (100%)	22 (91.6%)[h]	16 (66.6%)[i,j]	15 (62.5%)	13 (54.2%)
eAg(+)eAb(+)	0	1 (4.2%)[h]	4 (16.7%)[i]	5 (20.8%)	6 (25.0%)
eAg(−)eAb(+)	0	1 (4.2%)[h]	2 (8.4%)[j]	2 (8.4%)	2 (8.3%)
HBsAg(−)HBe(−)	0	0	2 (8.4%)	2 (8.4%)	3 (12.5%)
HBsAb(−)	0	0	1 (4.2%)	1 (4.2%)	2 (8.4%)
HBsAb(+)	0	0	1 (4.2%)	1 (4.2%)	1 (4.2%)

[a–j] $P < 0.01$

Table 2. Correlation among macrophage, T, B cell and serum HBVDNA, HBe, HBsAg markers and proteinuria in HBVMN patients

	HBsAg (+)			HBsAg (−)	
	eAg (+) eAb (−)	eAg (−) eAb (−)	eAg (−) eAb (+)	HBsAb (−)	HBsAb (+)
No. of cases	24	16	16	2	4
HBVDNA (+)					
in Monoclonal antibodies	15	0	0	0	0
T cell	23	3	0	0	0
B cell	21	1	0	0	0
in plasma	24	8	2	0	0
Proteinuria					
>40 mg/M²/hr	22	1	1	0	0
4–40 mg/M²/hr	2	6	2	0	0
<4 mg/M²/hr	0	9	13	2	4

were free from spurious DNA sequences of bacterial origin. With EcoRI digestion, only 3.2 kb HBV DNA was detected in macrophages, T cells and B cells before six months post-onset of disease. HindIII sites were not observed in the HBV DNA. These results indicate that the HBV DNA does not integrate into the genomic DNA of the host mononuclear cells but is present in a free replicating form.

The study of Yoffe et al also found that replicating forms of the HBV genome were present primarily in monocytes. Low levels of hybridization also were detected in B cells, whereas the T cell fraction appeared to be devoid of these replicating forms [9]. Our results demonstrated cellular HBV DNA not only in the macrophages and B cells, but also in the T cells. The different results may be due to the types of patients or the study models. Yoffe's patients had liver disease, and our cases demonstrated predominantly renal involvement. Yoffe only did one-time blood samples, while we did serial studies. The presence of HBV DNA in macrophages during the early stage of disease course and in T or B cells in HBVMN patients suggests that HBV is involved in immune regulation [10].

The study of Lai et al also demonstrated an increase of plasma HBV DNA concentration in HBVMN patients treated with corticosteroids [20]. Patients had a persistent elevation of HBeAg in their serum after corticosteroid withdrawal [20]. Therefore, the use of corticosteroids could lead to a potential risk of enhancing viral replication.

HBVMN is definitely not a benign disease. Some HBVMN patients have progressed into chronic renal failure [11]. It is necessary and important to evaluate the mechanism of HBVMN and to search for a satisfactory treatment in an area of high prevalence of HBV infection like Taiwan. It is notable that some of the patients with HBV infection have HBVMN while others do not. We hypothesize that: 1) patients with HBVMN probably have a defect in immune regulatory function and cannot produce high affinity antibodies to neutralize antigen present in the body; 2) a defect in the reticuloenthothelial system (RES) to clean circulating immune complexes may also be present in these patients. This disease in the past was treated by immune inhibitory drugs, which probably further inhibited the production of high affinity antibodies. Thymic factor was also used successfully in mainland China to treat patients who had heavy proteinuria unresponsive to steroid therapy. The possible mechanism of pharmacologic effect of thymic factor is promotion of T cell function through enhancing the production of cytokines [21], that is, immune modulation. To decrease urinary protein loss and obtain seroconversion in these patients, adenine arabinoside [22, 23], thymic factor [24] and interferon [25] have been used. None of these, however, had a satisfactory result. Therefore, we planned to combine adenine arabinoside with thymic factor (Thymostimulin) to treat children with HBVMN who had failed steroid treatment but still had persistent heavy proteinuria.

Heavy proteinuria in 19 cases (79.2%) from our presented data disappeared within three months after starting this protocol. Microscopic hematuria, however, persisted in all cases. Proteinuria reappeared in some cases during upper respiratory tract infection. Eight cases had hepatitis marker change. Two cases had HBe seroconversion with HBs seroconversion. Another two cases had HBsAg (+)/HBe (+) attained seroconversion and disappearance of both HBsAg and HBeAg. Therefore,

this treatment protocol is more effective in the improvement of proteinuria.

In conclusion, first, our results do not support the earlier notion suggested by Cadrobbi et al [26] that corticosteroids given at the onset of nephrotic syndrome are associated with a favorable outcome of the infection or the related renal disease [20]. Use of corticosteroids in HBVMN patients should be avoided. Corticosteroid treatment not only runs the risk of enhancing viral replication but also may result in producing more low-affinity antibodies and/or decreasing activity of the reticuloendothelial system, thus making the body fail to clear the invading antigen. It then causes more deposition of immune complexes in the basement membrane of the capillary wall of the glomerulus. Second, combination therapy with adenine arabinoside and Thymostimulin is more effective in the improvement of proteinuria. Further research is necessary to determine methods of providing hepatitis B seroconversion.

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